TRANSLATION

ru0765
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TEST REPORT

Chloroformic acid methyl ester

Inhalation toxicity in the flow through system

in male and female SPF Wistar rats

4-hour LC_{50}

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1. SUMMARY

The study of the inhalation toxicity of chloroformic acid methyl ester in male and female Wistar rats led to the following results.

Male animals LC_{50}: 51 ppm

Female animals LC_{50}: 53 ppm

On account of the mortality rate, no LC_{50} could be calculated.

The LC_{50} was determined by plotting on Probit probability paper.

Signs of toxicity consisted mainly of impaired motor functions, of respiratory disorders and disorders of the autonomous reactions.

The gross-pathological examination of the animals that died revealed changes of the lungs.

The cause of death detected by the histopathological examination was respiratory insufficiency due to toxic damage to the lung parenchyma with serum, plasma and erythrocyte diapedesis and damage to the bronchial epithelium (report of Professor Dr. K. H. Langer, pages 31 - 34).

Necropsy of the animals sacrificed at the end of the study did not reveal any obvious gross-pathological changes in most cases. Changes of the lungs were observed sporadically.

2. PRELIMINARY REMARKS

For the determination and assessment of the toxic properties of an inhalable material, such as a gas, volatile substance or aerosol, the determination of the acute inhalation toxicity is a first step. It provides information about a possible risk to health after inhalation during short-term periods. The results may also be used as a basis for classification and labeling. Furthermore, it provides important information for establishing test concentrations of subacute inhalation studies and allows conclusions to be drawn about the toxicological mode of action.

The present study was carried out taking into account the following test guideline adopted by the OECD:

OECD Guideline For Testing Of Chemicals, 403
"Acute Inhalation Toxicity", OECD 1981

This test was carried out in accordance with the principles of Good Laboratory Practice. No unforeseen events which might have impaired the quality or integrity of the present study were reported.

3. OVERVIEW

Study No.:	85.0678
Test substance:	Chloroformic acid methyl ester
Type of test:	Evaporation at 72°C; 4-hour LC_{50}
Species/sex:	SPF Wistar rats/male and female
Sponsor:	Hoechst Intermediates
Beginning of study:	October 30, 1985
End of study:	January 23, 1986

RESPONSIBILITY

Industrial toxicology:	Dr. WEIGAND
Study Director:	Dr. HOLLANDER
Department of Toxicology:	Dr. MAYER
Department of Toxicology and Pathology:	Professor Dr. LANGER
GLP Unit:	Pharmacist Harston
Testing facility and retention of records:	Pharmaceutical Research Toxicology HOECHST AKTIENGESELLSCHAFT Frankfurt am Main, Germany

4. MATERIAL AND METHOD

4.1 Test substance

Name:	Chloroformic acid methyl ester
Purity:	At least 96%
Impurities:	<0.03% phosgene 0.9% dimethyl carbonate
Appearance:	Colorless liquid
Storage:	At room temperature under the fume hood
Date of receipt of sample:	Nov. 22, 1985

4.2 Animal species and housing

Species:	Rat
Strain:	Hoe: WISKf (SPF71)
Origin:	HOECHST AG, Kastengrund, Germany, SPF breed
Age of the animals:	8 – 10 weeks
Body weight at beginning of test	
Males:	x = 179.3 g (= 100%) x min = 161.0 g (- 10.2%) x max = 198.0 g (+ 10.4%)
Females	x = 177.4 g (= 100%) x min = 164.0 g (- 7.6%) x max = 194.0 g (+ 9.4%)
Randomization:	According to plan Nos. Males: 585/85, 587/85, 666/85, 220/85 Females: 586/85, 605/85, 667/85, 221/85
Housing:	In fully air-conditioned rooms in Makrolon cages (type 4) on softwood granules in groups of 5 animals each
Room temperature:	22 ± 2°C
Rel. humidity:	55 ± 10%
Illumination period:	12 hours daily
Acclimatization:	At least 5 days
Diet:	Rat diet Altromin 1324 (AItromin-GmbH, Lage/Lippe, Germany); ad libitum
Water:	Tap water in plastic bottles; ad libitum
Identification of the animals:	Marking of the fur with KMn04 and numbering of the cages

4.3 Test groups

Group	Conc. in the exposure chamber in ppm chloroformic acid methyl ester	Number of animals Male	Female
1	35	5	5
2	45	5	5
3	57	5	5
4	73	5	5

4.4 Test procedure

4.4.1 Principle

The tests were carried out in a 2.25 m³ chamber of Rhema Labortechnik, Hofheim, Germany.
The exposure chamber was operated under dynamic conditions at a pressure of 0.8 mbar below atmospheric. A gas cleaning equipment was used for disposal.
During the 4-hour whole body exposure in the specific concentrations, the rats were kept singly in wire mesh cages.

4.4.2. Preparation of the inhalation atmosphere

Chloroformic acid methyl ester was continuously applied to a vaporizer head using a continuous infusion injector and evaporated at 72°C. The mixture of preparation and air was passed into the inhalation chamber through a hose line at an air flow of 800 l/h. The preparation was introduced into the chambers in two phases so that the concentration desired was achieved rapidly. After the build-up phase, the amount applied of the test substance and the air flow in the chambers were as follows:

		Build-up phase in minutes	Volume/air flow in the build-up phase per hour	Volume/air flow after the build-up phase per hour
Group I	35 ppm	4	6 ml/2 m³	2.00 ml/6 m³
Group II	45 ppm	3.5	7 ml/2 m³	3.00 ml/6 m³
Group III	57 ppm	2	12 ml/2 m³	3.75 ml/12 m³
Group IV	73 ppm	4	12 ml/2 m³	3.75 ml/6 m³

4.4.2 Examinations

During inhalation, the behavior of the animals was exactly observed and recorded. After exposure, the animals were subjected to a post-exposure observation period of 14 days and their behavior was checked twice daily.The rats were weighed on days 7 and 14 after inhalation.

One female of Group 3 (57 ppm) was kept under observation for a total of 5 weeks.

Fatally intoxicated animals were necropsied immediately and subjected to a gross-pathological examination. The lungs were removed, fixed in Carnoy or formalin and transferred to Experimental Pathology.

The surviving test animals were sacrificed by CO_2 at the end of the post-exposure observation period and were also assessed by gross pathology.

During the inhalations, CO, CO_2, O_2, humidity and temperature were continuously measured in the exposure chamber using an air monitoring system of Hartmann & Braun.

The concentrations of chloroformic acid methyl ester in the respiratory air were measured using a Miran 80 single beam photometer (Foxboro Analytical, South Norwalk, U.S.A.). A calibration curve was established for the measuring instrument.Control calibrations were carried out weekly.

During the inhalations, the concentrations of the test substance in the exposure chamber were determined every 15 minutes.

Moreover, analyses of chloroformic acid methyl ester in the exposure chamber compared with those of Miran 80 were carried out in the analytical laboratory, Professor Kelker. For this purpose, 10 liters of the chamber atmosphere were sucked through a gas sampling tube within 10 minutes during exposure. Sampling was carried out twice at an interval of about 120 minutes.

5. RESULTS

5.1 Lethality and LC_{50}

The following lethality rates were recorded under the test conditions described:

Conc. in the exposure chamber in ppm chloroformic acid methyl ester	Lethality		
	Males	Females	Total
35	0/5	0/5	0/10
45	0/5	0/5	0/10
57	5/5	3/5	8/10
73	5/5	5/5	10/10

The fatally intoxicated animals died from 380 minutes after the beginning of exposure up to 13 days after inhalation.

The following mean fatal concentration (LC_{50}) was determined by plotting on Probit probability paper.

Males
LC_{50}: 51 ppm

Females
LC_{50}: 53 ppm

For mortality times see Annex 6.1.

5.2. Clinical signs

The following clinical signs of toxicity were observed in relation to the concentration: Palpebral fissure narrowed or closed; increased grooming effort; squatting posture; accelerated, irregular and jerky respiration; gasping breathing; drowsiness; staggering movements; whimpering and crackling breathing noises; retracted flanks; high-legged gait; abdominal position; sneezing; piloerection.

The body weight gain was retarded in males and females after exposure, but most of the surviving animals had regained their initial weights at the end of the study.

For individual data of clinical signs and body weight gain see Annex 6.1.

5.3. Necropsy finding

The gross-pathological examination of the males and females that died revealed dark red to black lungs in most animals. When the lungs were dissected, foamy liquid issued. Reddish aqueous liquid was often observed in the thoracic cavity. In some animals, the gastrointestinal tract was distended and a reddish liquid was detected in the intestines.

Necropsy of the animals sacrificed at the end of the study did not reveal any gross-pathological abnormalities in most cases. Lungs with dark red foci were observed sporadically.

For individual data of the organ changes detected by gross pathology see Annex 6.1.

5.4. Histopathological examination

The histopathological examination showed that, regardless of the sex of the animals and of the dose, there were similar findings in the lungs which confirm the respiratory insufficiency and are ultimately the cause of the spontaneous death. The 4-hour exposure with chloroformic acid methyl ester led to an increase in permeability in the region of the alveolar septa and thus to damage to the bronchial epithelium. In the further course, mucous and in some cases also purulent bronchitis developed.
For details see report of Professor Dr. K. H. Langer of Apr. 2, 1986 (Annex, pages 31-34).

Dr. Ho./Pflg.

GLP Unit (Initialed)

Pharmaceutical Research Toxicology
HOECHST AKTIENGESELLSCHAFT

(Signed: Apr. 11, 1986*)
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_____
* Date on the original German report

6. ANNEX

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	35 ppm
Species/Sex:	Rats/male
O_2 in the exposure chamber:	20.6 vol.%
CO_2 in the exposure chamber:	500-800 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	18.3-19.6°C
Rel. humidity in the exposure chamber:	36.3-43.6%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	185	196	233
2	192	206	248
3	198	217	246
4	194	213	262
5	191	201	242

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	35 ppm
Exposure:	Flow through system
Species/Sex:	Rats/male

Signs of toxicity

Time after the beginning of study	20'	30'	93'	120'	135'	240'	460'	1 d	2 d	3 d	4d - 14d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
					No. of animals with symptoms						
Squatting posture	5	5	5	5	5	5	5	5			
Palpebral fissure closed	5	5	5	5	5	5	5				
Increased grooming effort		5									
Jerky respiration			5	5	5	5	5				
Drowsiness				5	5	5	5				
Staggering gait					5	5	5				
Sneezing								5	5	1	
Piloerection								5	5	1	
No clinical signs										4	5

Necropsy findings

	No. of animals	Findings
5 animals sacrificed at the end of study	5	No abnormalities detected by gross pathology

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	35 ppm
Species/Sex:	Rats/female
O_2 in the exposure chamber:	20.6 vol.%
CO_2 in the exposure chamber:	500-800 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	18.3-19.6°C
Rel. humidity in the exposure chamber:	36.3-43.6%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	182	187	197
2	186	199	211
3	183	184	198
4	184	180	186
5	190	200	207

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	35 ppm
Exposure:	Flow through system
Species/Sex:	Rats/female

Signs of toxicity

Time after the beginning of study	20'	30'	93'	120'	135'	240'	460'	1 d	2 d	3d - 14d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
No. of animals with symptoms										
Squatting posture	5	5	5	5	5	5	5	5		
Palpebral fissure closed	5	5	5	5	5	5	5			
Increased grooming effort		5								
Jerky respiration			5	5	5	5	5			
Drowsiness				5	5	5	5			
Staggering gait					5	5	5			
Sneezing								5	5	
Piloerection								5	5	
No clinical signs										5

Necropsy findings

	No. of animals	Findings
5 animals sacrificed at the end of study	5	No abnormalities detected by gross pathology

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	45 ppm
Species/Sex:	Rats/male
O_2 in the exposure chamber:	20.6 vol.%
CO_2 in the exposure chamber:	450-600 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	19.3-19.6°C
Rel. humidity in the exposure chamber:	40.3-42.2%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	182	200	229
2	184	198	231
3	196	212	244
4	183	200	223
5	190	219	249

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	45 ppm
Exposure:	Flow through system
Species/Sex:	Rats/male

Signs of toxicity

Time after the beginning of study	10'	20'	65'	90'	100'	240'	1 d	2d-6d	7d - 14d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	No. of animals with symptoms								
Palpebral fissure narrowed	5								
Palpebral fissure closed		5	5	5	5	5			
Increased grooming effort		5	5						
Squatting posture		5	5	5	5	5	5		
Jerky respiration			2	5	5	5			
Staggering gait					5	5			
Drowsiness					5	5	5		
Sneezing							5	5	
Piloerection							5		
No clinical signs									5

Necropsy findings

	No. of animals	Findings
5 animals sacrificed at the end of study	3	No abnormalities detected by gross pathology
	2	Lungs with dark red foci

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	45 ppm
Species/Sex:	Rats/female
O_2 in the exposure chamber:	20.6 vol.%
CO_2 in the exposure chamber:	450-600 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	19.3-19.6°C
Rel. humidity in the exposure chamber:	40.3-42.2%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	183	189	199
2	186	194	209
3	194	199	204
4	191	198	207
5	184	189	197

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	45 ppm
Exposure:	Flow through system
Species/Sex:	Rats/female

Signs of toxicity

Time after the beginning of study	10'	20'	65'	90'	100'	240'	1 d	2d-6d	7d - 14d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
No. of animals with symptoms									
Palpebral fissure narrowed	5								
Palpebral fissure closed		5	5	5	5	5			
Increased grooming effort		5	5						
Squatting posture		5	5	5	5	5	5		
Jerky respiration			1	3	5	5			
Staggering gait					5	5			
Drowsiness					5	5			
Sneezing							5	5	
Piloerection							5		
No clinical signs									5

Necropsy findings

	No. of animals	Findings
5 animals sacrificed at the end of study	3	No abnormalities detected by gross pathology
	2	Lungs with dark red foci

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	57 ppm
Species/Sex:	Rats/male
O_2 in the exposure chamber:	20.5-20.6 vol.%
CO_2 in the exposure chamber:	400-600 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	19.5-19.8°C
Rel. humidity in the exposure chamber:	47.9-49.4%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	161	Died 27 hours after beginning of study	
2	163	Died 30 hours after beginning of study	
3	161	Died 24 hours after beginning of study	
4	167	152	died 12 d after inhalation
5	169	Died 27 hours after beginning of study	

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	57 ppm
Exposure:	Flow through system
Species/Sex:	Rats/male

Signs of toxicity

Time after the beginning of study	10'	20'	55'	80'	100'	210'	230'	240'	1d*	1d**	1d***
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	3/5	4/5
					No. of animals with symptoms						
Palpebral fissure narrowed	5										
Palpebral fissure closed		5	5	5	5	5	5	5			
Increased grooming effort		5	5								
Squatting posture		5	5	5	5	5	5	5	4	2	1
Jerky respiration			2	5	5	4					
Staggering gait					5	5	5	5			
Drowsiness					5	5	5	5			
Gasping breathing						1	5	5	4	2	1
Red incrusted snout									4	2	1
Crackling-whimpering breathing noises									4	2	1
Piloerection									4	2	1

*7 hours **10 hours ***13 hours

Time after the beginning of study	2d	3d	4d	5d	6-9d	10d	11d	12d
Mortality rate	4/5	4/5	4/5	4/5	4/5	4/5	4/5	5/5
Squatting posture	1	1	1					
Red incrusted snout	1	1	1					
Crackling-whimpering breathing noises	1	1	1			1	1	
Piloerection	1	1	1	1			1	
Irregular respiration						1	1	
Retracted flanks							1	
High-legged gait							1	
No clinical signs					1			

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	57 ppm
Exposure:	Flow through system
Species/Sex:	Rats/male

Necropsy findings

	No. of animals	Findings
5 animals that died	1	Lungs dark red; issue of foamy liquid after dissection. Stomach and intestines distended; reddish liquid in the intestines
	1	Lungs dark red to black; issue of foamy liquid after dissection. Stomach and intestines distended; reddish liquid in the intestines
	1	Lungs dark red to black; issue of foamy liquid after dissection. Stomach and intestines distended; reddish liquid in the intestines
	1	Small lung dark red
	1	Lungs dark red; issue of foamy liquid after dissection. Stomach and intestines distended; reddish liquid in the intestines. Lung surface with irregular (1-5 mm) whitish yellow bulgings

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	57 ppm
Species/Sex:	Rats/female
O_2 in the exposure chamber:	20.5-20.6 vol.%
CO_2 in the exposure chamber:	400-600 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	19.5-19.8°C
Rel. humidity in the exposure chamber:	47.9-49.4%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d	21 d	28 d	35 d
1	168	166	194			
2	183	159	140	156	176	186
3	165	Died 28 hours after beginning of study				
4	168	156	died 13 d after inhalation			
5	173	Died 24 hours after beginning of study				

6.1 Individual findings

Study No.: 85.0810
Test substance: Chloroformic acid methyl ester
Concentration in the exposure chamber: 57 ppm
Exposure: Flow through system
Species/Sex: Rats/female

Signs of toxicity

Time after the beginning of study	10'	20'	55'	80'	100'	210'	230'	240'	1d*	1d**	2d	3d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	2/5	2/5
	No. of animals with symptoms											
Palpebral fissure narrowed	5											
Palpebral fissure closed		5	5	5	5	5	5	5				
Increased grooming effort		5	5									
Squatting posture		5	5	5	5	5	5	5	4	3	3	
Jerky respiration			1	5	5	4	4	4				
Staggering gait					5	5	5	5				
Drowsiness					5	5	5	5				
Gasping breathing						1	1	1	4	3		
Red incrusted snout									4	3	3	
Crackling-whimpering breathing noises									4	3	3	3
Piloerection									4	3	3	
Sneezing												3

*7 hours **11 hours

Time after the beginning of study	4d	5d-8d	9d	10d	11d	12d	13d	14d	15d-19d
Mortality rate	2/5	2/5	2/5	2/5	2/5	2/5	3/5	3/5	3/5
	No. of animals with symptoms								
Sneezing	3								
Calm			2	2					
Retracted flanks			2	2	2	2	1	1	1
Irregular respiration			1	2	2	2			
High-legged gait					2	2	1	1	1
Piloerection					2	2	1	1	1
Jerky respiration							1	1	1
No clinical signs		3	1	1	1	1	1	1	

Time after the beginning of study	20d	21d-25d	26d-35d
Mortality rate	2/5	2/5	3/5
	No. of animals with symptoms		
Retracted flanks	1	1	
Jerky respiration	1	1	1
Crackling-whimpering breathing noises		1	1

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	57 ppm
Exposure:	Flow through system
Species/Sex:	Rats/female

Necropsy findings

	No. of animals	Findings
3 animals that died	1	Lungs dark red; issue of foamy liquid after dissection. Stomach and intestines distended; reddish liquid in the thoracic cavity
	1	Lung surface with irregular (1-5 mm) whitish yellow bulgings; issue of foamy liquid after dissection. Stomach and intestines distended; reddish liquid in the intestines
	1	Partially eaten; autolysis
2 animals sacrificed at the end of study	1	Lungs seem to be distended and enlarged; pale pink with dark red foci, some lungs dark red
	1	No abnormalities detected by gross pathology

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	73 ppm
Species/Sex:	Rats/male
O_2 in the exposure chamber:	20.6 vol.%
CO_2 in the exposure chamber:	400-600 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	19.4-19.9°C
Rel. humidity in the exposure chamber:	45.8-49.1%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	172	Died 24 hours after beginning of study	
2	170	Died 380 min after beginning of study	
3	180	Died 24 hours after beginning of study	
4	174	Died 24 hours after beginning of study	
5	174	Died 410 min after beginning of study	

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	73 ppm
Exposure:	Flow through system
Species/Sex:	Rats/male

Signs of toxicity

Time after the beginning of study	8'	15'	20'	65'	85'	120'	147'	190'	220'	240'	380'	410'	1d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	2/5	5/5
	No. of animals with symptoms												
Palpebral fissure narrowed	5												
Palpebral fissure closed		5	5	5	5	5	5	5	5	5	4	3	
Increased grooming effort		5	5										
Squatting posture		5	5	5	5	5	5	5	5	5	4	3	
Jerky respiration			5	4	2	3	2						
Staggering gait					5	5	5	5	5	5	4	3	
Drowsiness					5	5	5	5	5	5			
Gasping breathing						1	2	3	5	5	4		
Crackling-whimpering breathing noises												3	

Necropsy findings

	No. of animals	Findings
5 animals that died	1	Lungs dark red; issue of aqueous liquid after dissection. Reddish aqueous liquid in the thoracic cavity
	1	Lungs dark red to black; issue of aqueous liquid after dissection
	1	Lungs dark red to black; issue of aqueous liquid after dissection. Reddish liquid in the thoracic cavity
	1	Lungs dark red to black; issue of aqueous liquid after dissection. Reddish aqueous liquid in the thoracic cavity
	1	Lungs dark red; issue of aqueous liquid after dissection

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	73 ppm
Species/Sex:	Rats/female
O_2 in the exposure chamber:	20.6 vol.%
CO_2 in the exposure chamber:	400-600 ppm
CO in the exposure chamber:	0 ppm
Temperature in the exposure chamber:	19.4-19.9°C
Rel. humidity in the exposure chamber:	45.8-49.1%

Body weight in g

Body weight during the test and observation periods

Animal No.	Day of exposure	Post-exposure observation period 7 d	14 d
1	165	Died 24 hours after beginning of study	
2	168	130	died 12 d after inhalation
3	166	Died 24 hours after beginning of study	
4	164	Died 2 d after inhalation	
5	164	Died 2 d after inhalation	

6.1 Individual findings

Study No.: 85.0810
Test substance: Chloroformic acid methyl ester
Concentration in the exposure chamber: 73 ppm
Exposure: Flow through system
Species/Sex: Rats/female

Signs of toxicity

Time after the beginning of study	8'	14'	15'	20'	55'	65'	85'	125'	240'	1 d	2 d	3 d	4 d
Mortality rate	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	4/5	4/5	4/5
	No. of animals with symptoms												
Palpebral fissure narrowed	4	5											
Palpebral fissure closed			5	5	5	5	5	5	5	3			
Increased grooming effort			5	5	5								
Squatting posture				5	5	5	5	5	5	3	1	1	1
Jerky respiration		1	1	1	2	5	5	4	4				1
Staggering gait							5	5	5				
Drowsiness							5	5	5				
Gasping breathing								1	1	3	1	1	
Red incrusted snout										3	1		
Crackling-whimpering breathing noises										3	1	1	
Piloerection											1	1	1

Time after the beginning of study	5d	6d	7d-10d	11d	12d*	12d**
Mortality rate	4/5	4/5	4/5	4/5	4/5	5/5
	No. of animals with symptoms					
Squatting posture	1	1	1			
Piloerection	1	1	1	1	1	
High-legged gait		1	1			
Accelerated respiration (200 breaths/min)		1	1	1	1	
Crackling breathing noises			1	1	1	
Abdominal position				1	1	
Tail cyanotic					1	

*7 hours **13 hours

6.1 Individual findings

Study No.:	85.0810
Test substance:	Chloroformic acid methyl ester
Concentration in the exposure chamber:	73 ppm
Exposure:	Flow through system
Species/Sex:	Rats/female

Necropsy findings

	No. of animals	Findings
5 animals that died	1	Lungs dark red; issue of aqueous liquid after dissection. Reddish aqueous liquid in the thoracic cavity
	1	Right lung dark red. Liver with spotted pale areas
	1	Lungs dark red to black; issue of aqueous liquid after dissection. Reddish aqueous liquid in the thoracic cavity
	1	Lungs dark red to black; autolysis
	1	Lungs dark red to black; autolysis

Experimental Pathology
Professor Dr. K. H. Langer
Apr. 2, 1986*

Report on the histopathological examination of rat lungs after inhalation of chloroformic acid methyl ester

Study No. 85.0810

The sections of rat lungs which were stained with HE, van Gieson's elastica, PAS, Berlin blue, Pearse or HBFP and some of which had been fixed in Carnoy and some of which in formalin were examined. The animals had been exposed in the inhalation test for 4 hours (whole body exposure) and died spontaneously at different times; one animal was sacrificed after 5 weeks.

Animals examined, survival periods and necropsy numbers:

57 ppm	1 male	27 hours	= S 303/86
	2 male	30 hours	= S 304/86
	3 male	24 hours	= S 305/86
	4 male	12 days	= S 306/86
	5 male	27 hours	= S 307/86
	2 female	5 weeks	= S 308/86
	3 female	28 hours	= S 309/86
	5 female	24 hours	= S 310/86
73 ppm	1 male	24 hours	= S 311/86
	2 male	380 min	= S 312/86
	3 male	24 hours	= S 313/86
	4 male	24 hours	= S 314/86
	5 male	410 min	= S 315/86
	1 female	24 hours	= S 316/86
	2 female	12 hours	= S 317/86
	3 female	24 hours	= S 318/86
	4 female	2 days	= S 319/86
	5 female	2 days	= S 320/86

Histopathological finding

Regardless of the sex of the animals and of the dose, the findings obtained in the lungs were similar; they showed only a certain range of variation among the animals with regard to severity, but they confirm the respiratory insufficiency and are thus the cause of the spontaneous death.

A more or less diffuse acute edema detected in all lobes examined, with thickening of the alveolar septa and with issue of low-protein liquid into the alveolar spaces, was the main finding; in some sections, there were signs of acute hyperinflation of alveolar spaces (acute emphysema), as observed in dyspnea or asphyxia. The adventitia of small and large vessels and the surroundings of branches of the bronchial tree were also widened and edematous. Furthermore, issues of fibrin and erythrocytes into alveolar spaces and necroses of the parenchyma with atelectasis were found; these were sometimes localized, sometimes they affected complete lobes or they were found extensively subpleurally. In the alveolar lumens, there was a large quantity of macrophages or rounded alveolar epithelial cells with storage of protein substances; the iron reaction was negative.

Necroses were sometimes also detected in the bronchial epithelium, but in some cases the bronchial epithelium showed an increased formation of mucus and there were changes in the form of mucous or mucopurulent bronchitis.

The changes mentioned were found in animals S 303 - 305, 307, 309 - 311, 313, 314, 317 and 318.

As exceptions, animals S 312 and 315 (380- and 410-minute survival period respectively) only revealed a practically diffuse edema with individual acutely hyperinflated lung sections.

Diffuse edema was also mainly found in animals S 316, 319 and 320 (24-hour or 2-day survival period), and there were also small issues of fibrin. Animal S 306 (12-day survival period) only revealed a focal acute edema in one lobe and signs of mucopurulent bronchitis – in addition to subpleural changes of the kind described above. In animal S 308 (sacrificed after 5 weeks), the severer changes detected were only very localized, i.e. their extension was clearly slighter; there was also mucous bronchitis.

Discussion

The 4-hour whole body exposure to chloroformic acid methyl ester led to lung changes which are regarded as increase in permeability in the region of the alveolar septa and as damage to the bronchial epithelium regardless of the concentration (57 or 73 ppm). As regards the increase in permeability, there was an issue of low-protein liquid during the first few hours after exposure; later, there were also issues of fibrin monomers and erythrocytes sometimes accompanied by parenchyma necroses and by the occurrence of macrophages phagocytizing protein. The latter findings may be accentuated subpleurally. The syndrome of mucous and sometimes also purulent bronchitis obviously develops only in the course of the disease, with the formation of mucus to be regarded as a reactive reaction.

Summary

The whole body exposure to 57 or 73 ppm of chloroformic acid methyl ester led to the death of most of the rats after 2 days at the latest as a result of respiratory insufficiency due to toxic damage to the lung parenchyma with serum, plasma and erythrocyte diapedesis and damage to the bronchial epithelium.

(Signed)
Professor Dr. K. H. Langer

Note:
If further studies are carried out,
1. the tissue should only be fixed in formalin and
2. tissue from the gastrointestinal tract – depending on the gross-pathological aspect – should also be preserved from some animals.

Prof.L/GL

To Dr. Hollander
cc Dr. Mayer

GLP Unit

Apr. 18, 1986*

Title: Chloroformic acid methyl ester
Inhalation toxicity in the flow through system
in male and female SPF Wistar rats
4-hour LC_{50}

Date: Apr. 11, 1986

Study No.: 85.0810

This study was inspected at regular intervals and written documents signed in accordance with the regulations were submitted to the management of the research facility and to the Study Director as follows:

Inspection	Report
Oct. 29, 1985	Oct. 29, 1985
Oct. 30, 1985	Oct. 30, 1985
Apr. 18, 1986	Apr. 18, 1986

Pharmaceutical Research
GLP Unit
(Signed)

* Date on the original German report